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Neurochemical correlates of conditioned circling within localized regions of the striatum

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Summary. Two experiments were conducted to determine the effects of conditioned circling on the concentrations of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) within discrete regions of the striatum (STR). The first study assessed the inherent regional distributions of these compounds with respect to the three primary axes: anterior-posterior, dorsal-ventral and medial-lateral. Concentrations of DA, DOPAC and HVA and the ratios of each metabolite to DA were found to vary across each dimension. However, the topographical distribution of each compound was unique. The results of the first experiment confirm that the STR is not a homogeneous structure. It is possible that the regional variations in dopamine metabolism underly the diverse functions which the STR is thought to modulate. The second experiment determined whether specific regions of the STR were differentially involved in the mediation of conditioned circling. DA metabolism, as estimated by metabolite concentrations and metabolite to DA ratios, was bilaterally increased within the anterior dorsomedial and dorsolateral STR, relative to non-circling, water-deprived controls. DOPAC and the corresponding ratio were enhanced selectively within the dorsomedial region, whereas HVA and its ratio to DA were increased preferentially within the dorsolateral STR. The ratio of DOPAC to DA was also enhanced within the anterior ventromedial STR. No other significant neurochemical effects were detected. These results support the hypothesis that the dorsal STR critically subserves circling. Moreover, it is possible that the medial and lateral regions of the dorsal STR are differentially involved in circling. These results also confirm previous reports of bilateral augmentation of striatal DA metabolism in association with high rates of conditioned circling.

Key words: Conditioned circling – Rotation – Dopamine – Metabolism – Striatum

Introduction

It is generally accepted that circling mediated by the nigrostriatal system is dependent upon an imbalance in dopamine (DA) activity within the two striata (STR). Moreover, it has been hypothesized that the direction of circling is dependent upon which STR is "dominant" (with respect to DA activity) and that the circling is directed away from the more active STR (cf. Pycock 1981 for review).

According to this hypothesis, it might be expected that animals trained to circle for reinforcement would exhibit increases in DA activity strictly within the contralateral STR. Indeed, Yamamoto and Freed (1982) have reported increased concentrations of both DA and 3,4-dihydroxyphenylacetic acid (DOPAC) within the contralateral STR of rats trained to circle for sucrose reinforcement. In contrast, however, other laboratories have only observed bilateral elevations in striatal DA metabolism in rats exhibiting high rates of conditioned circling (Schwarting and Huston 1987; Szostak et al. 1986).

A potential explanation for these discrepant results concerns the established functional heterogeneity of the STR (e.g. Dunnett et al. 1981a, b). It is possible that conditioned circling is modulated by restricted portions of the STR and that these regions are differentially involved. Joyce et al.

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(1981) have observed that infusions of DA into the dorsal STR result in strong contraversive circling (i.e. away from the site of infusion) whereas injections into ventral regions are associated with only weak contraversive circling. These findings have been extended by Wolfson and Brown (1983) who have demonstrated that rotation induced by intrastriatal DA injections is dependent upon the concentration of DA within the dorsal STR. Subsequent research by Joyce and van Hartesveldt (1984) has further indicated that it is the medial portion of dorsal STR that subserves rotation while postural deviation (i.e. behaviours directed to the environment ipsiversive or contraversive to the injection site) is selectively subserved by the lateral dorsal STR.

Conditioned circling may also be associated with decreases in DA metabolism within restricted striatal regions, the direction and magnitude of the neurochemical changes being dependent upon the side of the STR in relationship to the direction of turning (i.e. ipsiversive vs contraversive). Vaccarino and Franklin (1984) have suggested that the medial and lateral substantia nigra pars compacta (SNC) are functionally antagonistic with respect to their involvement in circling. Infusions of apomorphine into the medial SNC elicit ipsiversive circling, whereas similar injections into the lateral SNC are without effect. In contrast, infusions of alpha-flupenthixol, a DA receptor antagonist, are associated with contralateral rotation when injected medially, but produce ipsiversive circling when infused laterally.

The detection of such an intrastriatal interplay is precluded when overall striatal DA and metabolite concentrations are analyzed. Accordingly, the increases in striatal DA metabolism previously associated with conditioned circling may not accurately reflect the involvement of the STR in this behavior. For example, assuming that conditioned circling is dependent upon an imbalance in striatal DA activity, the bilateral increases previously observed could reflect increases within the contralateral dorsal STR *and* the ipsilateral ventral STR.

To test this hypothesis, two experiments were conducted. The first experiment examined the distribution of DA, DOPAC and HVA concentrations within the STR of untreated, control rats. The extent to which the concentrations of these three compounds and the ratios of the metabolites to DA varied along the dominant axes (anterior-posterior; medial-lateral; dorsal-ventral) was determined. In addition, interactions between these axes were assessed. The second experiment examined the extent to which DA metabolism within discrete sub-regions of the STR is differentially influenced by conditioned circling.

Experiment 1

Previous studies have indicated that DA concentrations decrease along the anterior-posterior axis (Beal and Martin 1985; Tassin et al. 1976). A similar pattern of distribution has been described for DA metabolites (Beal and Martin 1985). While these experiments have clearly demonstrated sub-regional variations in DA activity, they have not assessed more subtle regional differences. Beal and Martin (1985) have reported that concentrations of DA, DOPAC and HVA do not vary along the medial-lateral axis. However, their statistical treatment of the data did not permit assessment of potential interactions between the various axes.

The present experiment was designed so that the distributions of DA, DOPAC and HVA could be assessed using higher-order statistical tests that would be more sensitive to detecting the various gradients of concentrations and the interplay between these gradients. In addition, DOPAC : DA and HVA : DA ratios were analyzed in the same fashion as it has been suggested that the ratios can serve as a sensitive indicator of DA utilization (Lavielle et al. 1978).

Materials and methods

Subjects

Naive, male Sprague-Dawley rats (Charles River Laboratories) ($N = 17$) were used in the present experiment. Rats were housed individually in standard, suspended wire cages located within a temperature controlled room and were acclimatized to the laboratory for one week before the commencement of the epxeriment. During this time, a 12 h light-dark cycle was maintained (lights on: 8:00 h) and food and water were available *ad libitum* in the cages. Rats were handled daily.

Procedure

On the test days (8:30 – 11:30 a.m.), rats were removed from their home cages, taken to a separate room and decapitated. Brains were rapidly removed, a coronal cut was made slightly caudal to the optic chiasm and the anterior section frozen on a microtome. Three consecutive, 1 mm thick, coronal sections were obtained and placed on ice. The decussation of the corpus callosum served to define the rostral extent of the anterior slice, and corresponded approximately to level A8920 μ of Konig and Klippel (1963). The anterior borders of the second and third slices corresponded approximately to levels A7890 μ and A6860 μ, respectively. The two anterior slices were placed on ice so that the rostral surfaces were face up. Left and right STR were dissected from these two sections by initially making a horizontal cut at the level of the anterior commissure (Fig. 1). The dorsolateral borders were defined by the corpus callosum while the medial extent was defined by the lateral ventricles and septum. Each STR was then

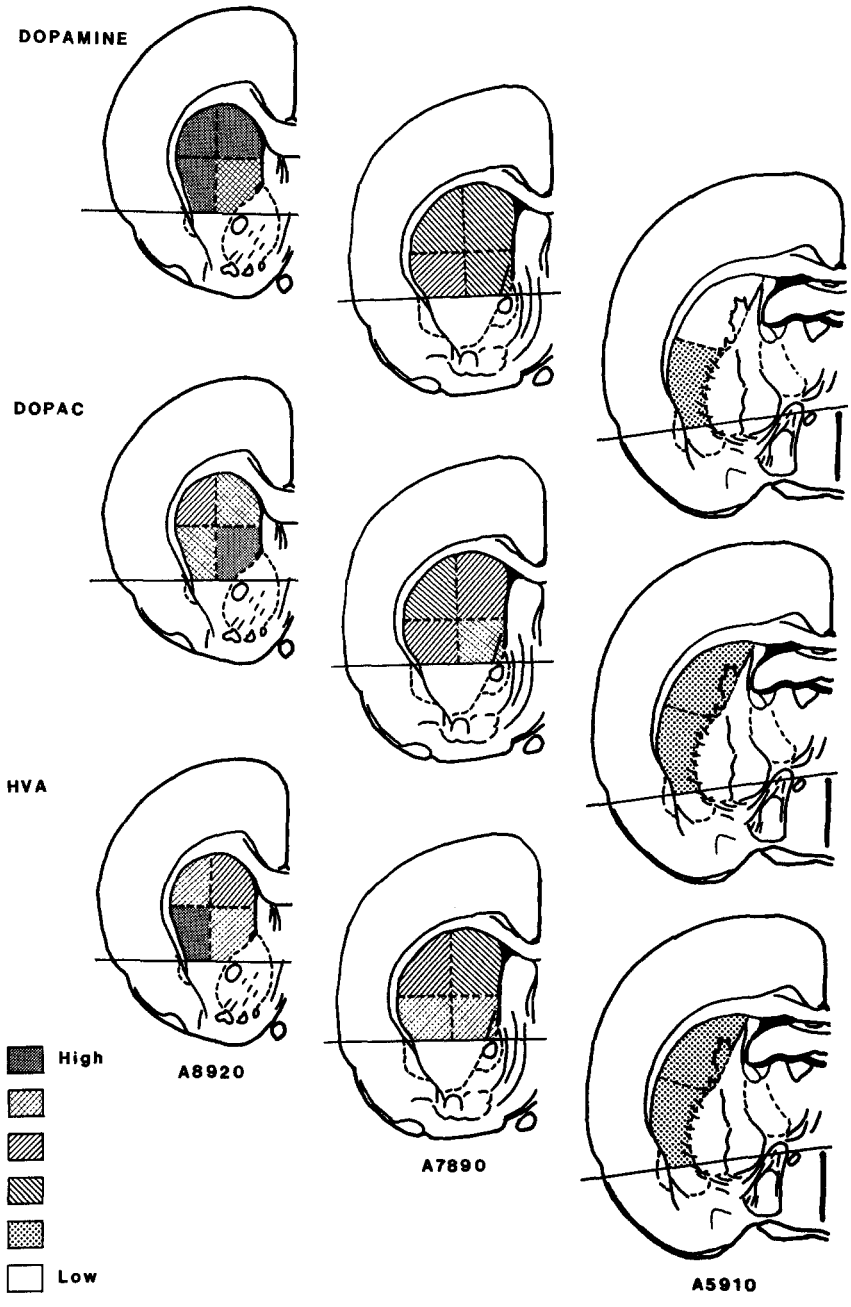

Fig. 1. Schematic representation of the distributions of DA, DOPAC and HVA within 10 sub-regions of the STR. Relative differences (high to low) in the intrastriatal distribution of each compound are depicted. The two anterior sections of the STR were divided into 4 quadrants while the tail of the STR was divided into two parts (see text for dissection procedures)

divided into 4 quadrants by making a horizontal and a vertical cut midway. The third slice was placed on ice so that the caudal surface was face up. A horizontal cut was made at the level of the fornix which served to define the ventral extent of the STR (Fig. 1). The medial border of the tail of the STR was defined by the globus pallidus whereas the corpus callosum marked the lateral limit. This structure was then divided into two equal parts by cutting midway along the dorsal/ventral axis. The tissue samples were immediately placed in 200 µl homogenizing solution and prepared for analysis of DA, DOPAC and HVA by high-pressure liquid chromotography with electrochemical detection (HPLC-ED), as described by Jakubovic et al. (1986). The concentration of each compound was expressed as nmol/g protein.

Statistical analyses

In order to determine whether there were any consistent left-right differences, one-way ANOVAs were conducted for each compound measured within each sub-region. To minimize the probability of making a Type I error, which would be increased due to the number of inter-dependent analyses conducted, the acceptable level of significance per comparison was set at 0.001 (Kirk 1968). As no consistent left-right differences were observed for any of the compounds or ratios, left and right values were pooled and the mean of the two sections used for purposes of assessing regional variations. The 4 regions within the anterior slice were initially compared with the corresponding areas obtained from the second

coronal section (i.e. posterior). The third section, containing the tail of the STR, was not included in this analysis as comparable sub-regions were not obtained. Accordingly, three-way ANOVAs, with each factor being a repeated measure having two levels (anterior/posterior; dorsal/ventral; medial/lateral), were conducted for each compound and ratio. Unique error terms were used in determining the significance of each main effect and interaction term (Kirk 1968). Tests of simple interactions and pair-wise comparisons (Newman-Keuls) were conducted when appropriate in order to identify the source of significant two-way and three-way interactions. The acceptable level of significance was set at 0.01 for all overall analyses and subsequent comparisons.

To determine whether concentrations varied within the tail of the STR, one-way ANOVAs with repeated measures (dorsomedial vs ventrolateral) were conducted for each compound and ratio. In addition, the existence of anterior-posterior gradients was further assessed by comparing the overall concentrations of each compound within the tail of the STR (i.e. mean of its two sub-regions) with the average concentrations found in each of the other two coronal sections of the STR. ANOVAs with one repeated measure (anterior vs posterior vs tail) were conducted for each compound and ratio. The source of significant main effects was identified using Newman-Keuls pair-wise comparisons.

In cases of incomplete data sets due to sporadic contamination of HVA samples, the data for that subject were eliminated from the overall analysis of HVA concentrations and HVA : DA ratios.

Results and discussion

Dopamine

Analysis of DA concentrations yielded a significant 3-way interaction: $F(1,16) = 8.95$, $P = 0.009$ (see Table 1). In order to identify the nature of this interaction, tests of the simple interactions composing the 3-way term were conducted.

Analysis of the AP × DV interaction within the lateral STR indicated that the anterior STR contained more DA than the posterior STR, irrespective of the dorsal-ventral axis: $F(1,16) = 91.16$, $P < 0.001$. Concentrations were also greater in the ventrolateral STR, relative to the dorsolateral STR: $F(1,16) = 9.30$, $P < 0.01$. Within the medial STR, the AP × DV interaction term was significant: $F(1,16) = 24.45$, $P < 0.001$. Pair-wise comparisons revealed that the anterior-posterior gradient was only significant within the dorsomedial STR and not in the ventromedial STR although a trend in the same direction was evident ($0.05 > P > 0.01$). Within the anteromedial STR, DA concentrations were greater in the dorsal region than in the ventral region. In contrast, no differences between dorsal and ventral concentrations were evident within the posteromedial STR.

Analysis of the AP × ML simple interactions yielded significant main effects of the AP axis, indicating that concentrations were greatest in the rostral regions of the STR, relative to more caudal regions: F's (1,16) = 181.51 and 43.08, P's < 0.001, for dorsal and ventral regions respectively. No medial-lateral differences were detected within the dorsal STR. However, a medial-lateral gradient was evident within the ventral STR: $F(1,16) = 21.42$, $P < 0.001$. Specifically, concentrations within the ventrolateral STR were greater than those detected within the ventromedial STR.

Analysis of the DV × ML interaction within the anterior STR yielded a significant interaction: $F(1,16) = 25.53$, $P < 0.001$. Pair-wise comparisons indicated that concentrations were higher in the anterodorsomedial STR, relative to those obtained within the anteroventromedial STR. No dorsal-ventral differences were detected within the anterolateral region of the STR. The interaction also reflected a medial-lateral gradient within the anteroventral STR, with concentrations being highest laterally. In contrast, analysis of this interaction within the posterior STR yielded only an effect of the dorsal-ventral axis with concentrations being greatest within the posteroventral STR: $F(1,16) = 9.24$, $P < 0.01$.

Analysis of DA concentrations within the tail of the STR indicated that concentrations were greatest within the ventrolateral tail, relative to the dorsomedial region: $F(1,15) = 37.80$, $P < 0.001$.

Table 1. Mean (± S.E.M.) concentrations (nmol/g protein) of DA, DOPAC and HVA within discrete sub-regions of the striatum

DA concentrations	Dorsal Lateral	Medial	Ventral Lateral	Medial
Anterior	851.09	913.56	878.91	770.29
	24.83	20.79	23.06	20.69
Posterior	663.00	675.79	739.71	702.03
	23.48	14.32	19.90	39.11
Tail		336.69	424.09	
		8.90	15.15	
DOPAC concentrations	**Dorsal Lateral**	**Medial**	**Ventral Lateral**	**Medial**
Anterior	133.15	170.24	171.18	202.38
	6.82	6.59	7.04	7.58
Posterior	116.56	130.41	126.94	163.06
	5.96	3.43	5.44	8.82
Tail		77.47	86.98	
		2.71	2.51	
HVA concentrations	**Dorsal Lateral**	**Medial**	**Ventral Lateral**	**Medial**
Anterior	62.97	54.46	77.43	64.89
	3.39	2.01	4.08	4.18
Posterior	53.42	46.69	68.71	67.84
	3.02	3.26	4.97	3.13
Tail		32.43	37.73	
		2.51	1.71	

The observation of the anterior-posterior gradient was confirmed and extended when overall DA concentrations within the tail were compared with the average concentrations obtained within the anterior and posterior sections: $F(1,15) = 320.01$, $P < 0.001$. Pair-wise comparisons indicated a progressive decrease in concentrations from anterior to caudal sections.

These results confirm earlier reports that DA concentrations vary along the rostral-caudal axis of the STR (e.g. Beal and Martin 1985; Tassin et al. 1976). Specifically, concentrations are lower in the more caudal regions. This gradient is apparent throughout the STR, that is to say, irrespective of any medial-lateral or dorsal-ventral differences. The present results also indicated that DA concentrations vary across the other axes. However, the medial-lateral and dorsal-ventral gradients are not as robust as the anterior-posterior gradient in that the direction and magnitude of these gradients vary across striatal sub-regions. In general, concentrations are greater in the ventral regions of the STR, relative to dorsal regions. One exception is the anterior-medial STR. In this area, the gradient is reversed with concentrations being greatest in the dorsal aspect of the anterior-medial STR. The lateral extent of the ventral STR is also richer in DA than is the medial counterpart. In contrast, no medial-lateral differences are discernible within the dorsal STR. In addition, concentrations within the tail of the STR varied such that the ventrolateral region contained more DA than did the dorsomedial area of the tail.

DOPAC and HVA

The 3-way ANOVA conducted upon DOPAC concentrations yielded a significant AP × DV interaction: $F(1,16) = 9.71$, $P < 0.01$. Pair-wise comparisons conducted to identify the source of this interaction indicated that DOPAC concentrations varied along the anterior-posterior gradient. Specifically, concentrations were highest in the anterior STR, irrespective of the dorsal-ventral axis. In addition, the ventral region contained more DOPAC than the dorsal aspect of both the anterior and posterior STR. The overall analysis also revealed a significant main effect of the medial-lateral axis, reflecting greater concentrations within the medial STR: $F(1,16) = 198.15$, $P < 0.001$.

DOPAC concentrations were greater in the ventrolateral region of the tail of the STR, relative to the dorsomedial area: $F(1,15) = 13.10$, $P = 0.003$.

Analysis of HVA concentrations indicated that all 3 main effects were significant: AP – $F(1,7) = 13.20$, $P = 0.008$; DV – $F(1,7) = 52.29$, $P < 0.001$; ML – $F(1,7) = 19.13$, $P = 0.003$. That is, concentrations of HVA were greatest in the anterior STR, relative to the posterior region. The dorsal-ventral gradient reflected increasing concentrations ventrally. Finally, concentrations were higher laterally than medially.

Sub-regional variations in HVA concentrations were not detected within the tail of the STR.

Comparison of overall concentrations of DOPAC and HVA within the tail of the STR with those detected in the anterior and posterior sections confirmed the existence of the anterior-posterior gradient for both metabolites: $F(1,15) = 189.90$, $P < 0.001$; $F(1,5) = 194.45$, $P < 0.001$, respectively. Pair-wise comparisons revealed that the anterior section had more DOPAC and HVA than did the posterior section. Concentrations within the tail were also less than obtained in the other two regions.

It should be noted that the pattern of distribution of HVA was very similar to that of DOPAC. Concentrations of both metabolites were greatest within the anterior region of the STR, relative to the posterior region and the tail of the STR. A ventral to dorsal decreasing gradient was also observed for both metabolites. However, the pattern of distribution for the two metabolites differed with respect to the medial-lateral axis. Specifically, DOPAC concentrations were highest in the medial STR whereas a gradient in the opposite direction was detected for HVA. While the significance of this observation is not immediately clear, it suggests that DA metabolism may vary within different regions of the STR. It is interesting to note that DA D_2 receptors also exhibit a medial to lateral increasing gradient (Joyce and Marshall 1987; Joyce et al. 1985). It is possible that a direct relationship exists between the distribution of D_2 receptors and DA metabolism.

DOPAC : DA and HVA : DA

The overall analysis of DOPAC : DA ratios yielded a significant AP × DV interaction: $F(1,16) = 38.23$, $P < 0.001$ (Fig. 2). Pair-wise comparisons indicated that within the dorsal STR, utilization of DA (as estimated by DOPAC : DA ratios) does not vary along the anterior-posterior axis. However, metabolism is augmented within the anteroventral STR, relative to that observed within the posteroventral STR. A dorsal-ventral gradient was detected within the anterior STR (metabolism being greatest ventrally). A similar gradient was not observed within the posterior STR. DA metabolism was also found to vary along the medial-lateral axis: $F(1,16) = 198.15$,

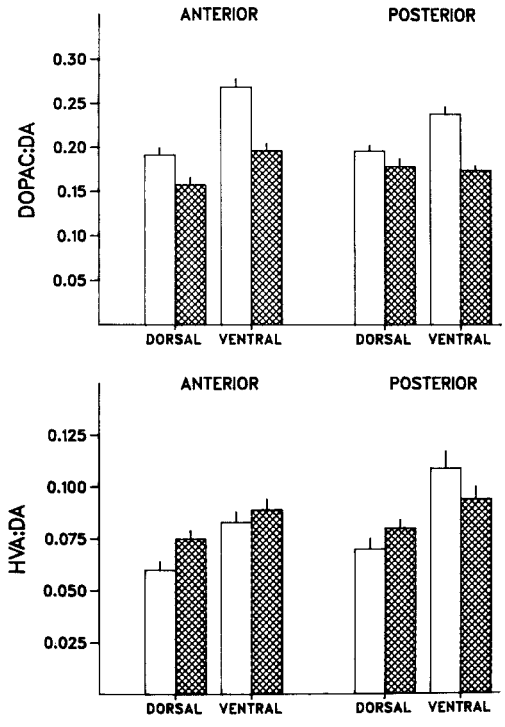

Fig. 2. Ratios of DOPAC and HVA to DA in 8 sub-regions of the striatum. Values represent the mean (± S.E.M.) ratios. □ Medial; ■ lateral

$P < 0.001$. Specifically, ratios of DOPAC to DA were greatest within the medial STR.

The DOPAC : DA ratio was higher in the dorsomedial tail of the STR, relative to the ventrolateral region of the tail: $F(1,15) = 19.50$, $P = 0.001$.

Overall analysis of the HVA : DA ratios yielded a significant AP × ML interaction: $F(1,7) = 12.08$, $P = 0.01$ (Fig. 2). Pair-wise comparisons conducted upon the means comprising this effect indicated that metabolism was enhanced within the posteromedial STR relative to the anteromedial STR. No other comparisons within this interaction were significant. The overall analysis also revealed that metabolism was greater within the ventral STR when compared to the dorsal STR: $F(1,7) = 42.30$, $P < 0.001$. No regional differences were detected within the tail of the STR.

Analysis of both DOPAC : DA and HVA : DA ratios within the anterior, posterior and tail regions of the STR failed to reveal any significant differences.

To summarize, an anterior-posterior gradient was observed in restricted regions of the STR for both DOPAC : DA and HVA : DA ratios (i.e. DOPAC : DA – ventral STR; HVA : DA – medial STR). A dorsal-ventral gradient was evident throughout the STR with DOPAC : DA and HVA : DA ratios being highest in the ventral regions of the STR. A medial-lateral gradient was found for DOPAC : DA ratios but not for HVA : DA.

The occurrence of regional differences in DOPAC : DA and HVA : DA ratios demonstrates that the topographical distributions of DA and its two primary metabolites are not identical (Fig. 1). For example, anterior-posterior gradients were evident for each compound. The existence of an anterior-posterior gradient for both ratios, within restricted regions of the STR, indicates that the slopes of this gradient for each compound are not identical throughout the STR. This indicates that there may exist regional differences in the metabolism of DA in terms of rate and, perhaps, mechanism.

Experiment 2

The diverse regional variations in DA metabolism observed in the first experiment provide further evidence for the functional heterogeneity of the STR. Given this heterogeneity, it is conceivable that DA metabolism within different striatal regions is not uniformly affected during the execution of a given behavior. As previously indicated, high rates of conditioned circling have been associated with bilateral augmentation of striatal DA metabolism (Schwarting and Huston 1987; Szostak et al. 1986). It is possible, given the regional variations in DA metabolism, that these overall changes do not accurately portray the role of the STR in conditioned circling. Several independent lines of research have suggested that the dorsal STR may play a greater role in circling than other regions of the STR (Dunnett et al. 1981a; Joyce et al. 1981; Wolfson and Brown 1983). It has also been suggested that circling may be a consequence of specific parts of the nigrostriatal system functioning in a mutually antagonistic fashion (Vaccarino and Franklin 1984). The extent to which various regions of the STR are differentially involved in conditioned circling was assessed in the second experiment.

Material and methods

Subjects and apparatus

Naive, male Sprague-Dawley rats (Charles River Laboratories), as described in Experiment 1, were placed on a food deprivation schedule that resulted in their being maintained at 85–90% of their ad libitum weights. A food deprivation regimen was employed as it had been observed that animals would acquire the conditioned circling response, when food was used as the reinforcing stimulus, without becoming dehydrated or exhibiting severe weight loss

(Szostak et al. 1988a). For the final phases of training and testing, rats were given restricted access to water while food was once again available freely in their home cages. This change in procedure was necessitated by the observation of bilateral increases in DOPAC and HVA in non-circling rats that had consumed the same amount of food as received by animals that were trained to circle for food (Szostak et al.1988a).

All behavioral testing was conducted in a plastic, cylindrical apparatus (diameter – 26 cm; height – 30 cm) equipped with a manually operated, motor-driven food hopper. During the final phase of testing, the food hopper was replaced with a motor-driven water dispenser (Skinner Electric Valve Division) calibrated to deliver 0.07–0.09 ml of water per reinforcement. Reinforcement (both food and water) was delivered into a trough located at the perimeter of the apparatus, 4 cm from the floor.

Procedure

Pre-screening. The directional bias of each subject was determined using a non-differential reinforcement paradigm whereby each complete rotation emitted was reinforced, regardless of its direction. The number of circles emitted in each direction during a 20 min test session was recorded. Directional bias was determined by dividing the number of rotations to the left by the total number of rotations.

Training. Half of the rats ($N = 9$) were trained to circle in their preferred direction for food reinforcement. Circling was initially established using a continuous reinforcement schedule, wherein each complete rotation (i.e. 360°), performed in the appropriate direction, was reinforced. Following 3–6 daily 20 min training sessions, the schedule of reinforcement was switched to a Fixed-Ratio 2 (FR-2), whereby every second rotation was reinforced. On the tenth FR-2 session, water (0.07–0.09 ml/reinforcement) was substituted for food as the reinforcer. Each rat received 6 daily 20 min training sessions during which circling was reinforced with water according to a FR-2 schedule of reinforcement. All training and subsequent test sessions were conducted between 8:30 a.m. and 12:30 p.m.

The remaining subjects ($N = 9$) were maintained on similar schedules of food and water deprivation and served as non-circling controls. Each subject was handled daily.

Testing. Upon attainment of stable rates of circling, rats received a single test session of 20 min during which time experimental contingencies were as described above. Immediately following the test session, rats were decapitated and their brains quickly removed. A coronal cut was made slightly anterior to the optic chiasm and the anterior section frozen on a microtome. The STR was removed and dissected as described in Experiment 1 (Fig. 1). The tissue samples were immediately placed in 200 µl homogenizing solution and prepared for analysis of DA, DOPAC and HVA by high-pressure liquid chromotography with electrochemical detection, as described by Jakubovic et al. (1986).

Statistical analyses

Each region was analysed separately as incomplete data sets, due to sporadic sample contamination, precluded the use of higher-order statistical designs. Accordingly, each analysis consisted of one between subject factor (Group) and one within subject factor (Hemisphere). The acceptable level of significance was set at $\alpha \leq 0.01$.

Table 2. Mean (± S.E.M.) concentrations (nmol/g protein) of DA, DOPAC and HVA within discrete sub-regions of the anterior striatum of water-deprived rats and rats trained to circle for water reinforcement

DA concentrations	Dorsal Lateral	Medial	Ventral Lateral	Medial
Controls	982.29	1062.67	1103.75	921.83
	50.15	26.64	46.92	74.59
Circlers	975.89	1055.28	1052.71	868.83
	50.14	29.72	42.00	54.11
DOPAC concentrations	**Dorsal Lateral**	**Medial**	**Ventral Lateral**	**Medial**
Controls	117.39	147.58	184.42	182.75
	4.90	3.53	7.28	14.84
Circlers	143.39	180.39	205.71	221.67
	7.84	7.62	11.12	11.79
HVA concentrations	**Dorsal Lateral**	**Medial**	**Ventral Lateral**	**Medial**
Controls	71.74	60.53	95.43	75.71
	3.22	4.96	6.64	4.92
Circlers	88.72	71.45	105.94	95.56
	3.25	5.71	5.91	8.82

Results and Discussion

Behavior

All rats exhibited stable levels of responding for water reinforcement following 6–7 training sessions. On the test day, the mean total number of rotations emitted was 230.22 (± 5.52).

Neurochemistry

Statistical analyses failed to reveal any significant effects of Hemisphere or any interactions between the Group and Hemisphere factors. In addition, concentrations of DA were not influenced by circling in any of the sub-regions assessed. Of the 10 regions that were examined, only the 4 anterior regions exhibited statistically significant changes in metabolite concentrations and/or ratios of the metabolites to DA (see Tables 2–4).

Within the anterior dorsolateral STR, HVA concentrations and the ratios of both HVA and DOPAC to DA were increased in rats trained to circle: F's(1,14) = 13.290, 9.384, 10.597; P's = 0.003, 0.008, 0.006, respectively (see Table 2; Fig. 3). A similar trend was observed when absolute concentrations of DOPAC were analyzed ($P = 0.02$). Within the anterior dorsomedial STR,

Table 3. Mean (± S.E.M.) concentrations (nmol/g protein) of DA, DOPAC and HVA within discrete sub-regions of the posterior striatum of water-deprived rats and rats trained to circle for water reinforcement

DA concentrations	Dorsal Lateral	Medial	Ventral Lateral	Medial
Controls	716.00	610.71	686.00	565.00
	32.19	31.89	37.68	56.63
Circlers	734.43	677.50	626.50	565.56
	19.55	37.59	44.91	30.75
DOPAC concentrations	**Dorsal Lateral**	**Medial**	**Ventral Lateral**	**Medial**
Controls	137.00	152.43	159.58	132.40
	5.54	10.13	4.21	13.24
Circlers	148.86	154.67	170.20	136.81
	8.82	8.50	16.06	8.06
HVA concentrations	**Dorsal Lateral**	**Medial**	**Ventral Lateral**	**Medial**
Controls	67.07	55.79	71.49	51.43
	2.60	3.49	2.80	6.92
Circlers	79.75	60.90	69.60	56.52
	4.34	2.38	6.58	3.30

Table 4. Mean (± S.E.M.) concentrations (nmol/g protein) of DA, DOPAC and HVA within discrete sub-regions of the tail of the striatum of water-deprived rats and rats trained to circle for water reinforcement

	Dorsomedial	Ventrolateral
DA concentrations		
Controls	434.83	567.78
	27.67	21.37
Circlers	501.22	537.83
	28.70	20.12
DOPAC concentrations		
Controls	81.05	95.02
	9.57	8.33
Circlers	90.09	95.09
	4.47	5.59
HVA concentrations		
Controls	35.58	44.91
	4.02	4.06
Circlers	41.62	50.12
	2.55	1.97

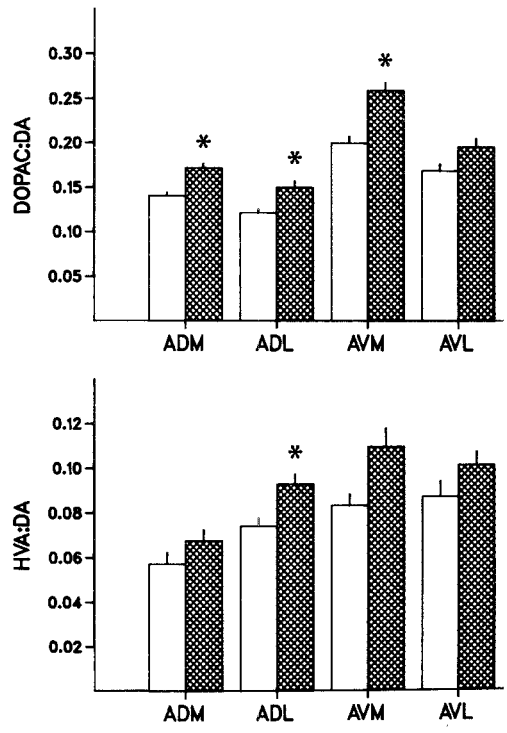

Fig. 3. Ratios of DOPAC and HVA to DA in anterodorsomedial (ADM), anterodorsolateral (ADL), anteroventromedial (AVM) and anteroventrolateral (AVL) striatum of rats trained to circle for water reinforcement and water-deprived controls. Values represent the mean (± S.E.M.) ratios. Rats were either tested for 20 min (circlers) or given access to water in their home cage for 20 min (controls) before being decapitated. * Differs from respective control value. □ Control; ■ circlers

only DOPAC concentrations and the ratio of DOPAC to DA were augmented bilaterally in rats trained to circle: $F(1,13) = 11.061$, $P = 0.005$; $F(1,13) = 21.998$, $P < 0.001$, respectively. The differential sensitivities of DOPAC and HVA in the anterior dorsomedial and dorsolateral regions of the STR may reflect the differential distributions of these two metabolites. In Experiment 1, it was observed that HVA concentrations were greater within the lateral striatal regions when compared to the medial extent. The reverse relationship was observed with DOPAC. In the present experiment, DOPAC was selectively increased within the dorsomedial region – the area that contains proportionately more DOPAC than HVA. Concurrently, HVA was preferentially increased in the dorsolateral region which contains proportionately more HVA than DOPAC. As previously suggested, the different patterns of distribution may reflect different metabolic processes. It is also possible that these two regions are also involved in different aspects of the circling response. (Joyce and van Hartesveldt 1984).

In contrast to the effects observed in the dorsal regions of the anterior STR, only limited differences were observed in the ventral striatal areas. Conditioned circling was associated with a bilateral increase in DOPAC : DA ratios within the ventromedial STR: $F(1,13) = 23.117$, $P < 0.001$. A similar trend was observed when the HVA : DA was

analyzed ($P = 0.03$). No significant differences were observed for any of the dependent measures in the ventrolateral region of the STR although there was a trend for DOPAC : DA to be bilaterally increased in animals trained to circle ($P = 0.05$).

These results confirm previous reports of bilateral augmentation of DA metabolism within the STR (Schwarting and Huston 1987; Szostak et al. 1986). The changes were bilateral despite training rats to circle in their preferred direction, a procedure which should enhance the likelihood of detecting a neurochemical asymmetry (Glick 1982; Robinson and Becker 1983). It should, thus, be noted that the present results fail to confirm the report by Yamamoto and Freed (1982) that conditioned circling is associated with an asymmetrical increase in striatal DA metabolism.

The localization of the neurochemical changes are consistent with the hypothesis that the dorsal STR is critically involved in circling (Joyce and van Hartesveldt 1984; Joyce et al. 1981; Wolfson and Brown 1983). However, changes were restricted to the anterior pole of the dorsal STR. It should be noted that previous experiments have not compared anterior dorsal to posterior dorsal STR. Thus, it appears that conditioned circling, as well as drug-induced circling, is mediated by a restricted region of the STR. Further experiments are required to determine whether the anterior dorsal STR plays a critical role in the mediation of circling in general or if its involvement is dependent upon the paradigm employed. In this regard, it should be noted that Dunnett and Iversen (1982) failed to find an effect of locus of lesion on the occurrence of subsequent drug-induced rotation. Thus, it is possible that the neural substrate of circling is dependent upon the method by which circling is induced.

General discussion

The results of the first experiment demonstrate that the striatal DA system is very complex with respect to the topographic distribution of DA and its two primary metabolites. In general, an anterior-posterior gradient was detected for each of the compounds examined, irrespective of medial-lateral and dorsal-ventral differences. This finding confirms and extends previous reports on the topographical distribution of DA and its metabolites (Beal and Martin 1985; Tassin et al. 1976). Concentrations of DA, DOPAC and HVA were also found to vary as a function of the dorsal-ventral axis. However, the direction of this gradient was found to depend upon the compound *and* the specific locus within the STR. In general, concentrations were higher within the ventral regions of the STR. One exception was observed within the anteromedial STR where DA concentrations were actually higher in the dorsal area than in the ventral region. Differences were also noted along the medial-lateral axis for each compound. The direction and the magnitude of DA's medial-lateral gradient were dependent upon the precise anterior-posterior locus. Concentrations were lower within the ventromedial STR when compared with the ventrolateral STR. This gradient was especially prominent within the anteroventral region of the STR. In contrast, no medial-lateral differences were detected within the dorsal STR. DOPAC concentrations were greatest within the medial STR, regardless of the anterior-posterior or dorsal-ventral axes. The highest concentrations of HVA were, in contrast, found laterally. Again, the medial-lateral gradient did not vary as a function of the other axes.

Previous evidence for either dorsal-ventral or medial-lateral gradients for DA, DOPAC and HVA has been inconsistent. For example, Beal and Martin (1985) and Tassin et al. (1976) did not detect either gradient for any of the compounds. In contrast, Di Paolo et al. (1982) reported regional variations within a given coronal section. Joyce et al. (1985) reported that DOPAC and HVA concentrations were higher in the ventral STR, relative to the dorsal STR. These differences were also evident when metabolite to DA ratios were examined. The results of Experiment 1 confirm the latter observations. It is possible that the discrepancies within the literature reflect differences in both experimental design and statistical treatment of data. The present experiment used experimental procedures and statistical methods that would permit the detection of axial variations, as well as interactions between the axes.

The significance of the various topographical distributions observed for DA, DOPAC and HVA is perhaps best understood in terms of the regional variations in DA utilization, as estimated by metabolite to DA ratios. For example, the anterior-posterior gradients were found to vary as a function of the other axes. Such a gradient was only evident within the ventral STR for the DOPAC : DA ratio and only within the medial STR for the HVA : DA ratios. Both ratios also exhibited dorsal-ventral differences, with metabolism being higher within the ventral region of the STR. The medial-lateral gradient detected with DOPAC concentrations was confirmed when the DOPAC : DA ratios were analyzed. Despite the existence of a lateral-medial gradient in HVA concentrations, no differences were detected when HVA : DA ratios were examined. The fact that the topographical distributions of the ratios are

not the same as the distribution patterns described for the specific metabolites or for DA indicates that the regional variations are not proportionate across compounds in all of the sub-regions.

The complexity of the striatal DA system is further illustrated by considering the topographical distribution of DA receptors. Joyce and associates have reported that DA D_2 receptors show a lateral to medial gradient within the STR, and are without any obvious anterior-posterior or dorsal-ventral gradients (Joyce et al. 1985; Joyce and Marshall 1987). It is interesting to note that the distribution of D_2 receptors corresponds with the lateral-medial gradient exhibited by the metabolite HVA. The significance of this association is not clear at this time.

Taken together, the results of the first experiment clearly indicate that the striatal dopaminergic system is *not* a unitary system. Complex regional variations in concentrations of DA and both metabolites are evident. It is particularly important to note that the topographical distribution of *each compound* is unique, suggesting that DA may be released and metabolized differentially within discrete regions of the STR. This realization may facilitate understanding of the neural basis of the functional heterogeneity of the STR. Specifically, it may account for the fact that a variety of behaviors known to be modulated by DA are differentially sensitive to pharmacological interventions (e.g. Tombaugh et al. 1983).

The results of the second experiment further support the view that the STR is functionally heterogeneous. As previously reported, high rates of conditioned circling have been associated with bilateral increases in DA metabolism (Schwarting and Huston 1987; Szostak et al. 1986). However, the second experiment demonstrated that these changes are not observed throughout the STR. Rather, increases in DA metabolism are restricted to the anterior portion of the STR, and in particular, within the dorsal aspect of the anterior STR.

The location of these changes is consistent with reports by Joyce and associates (Joyce et al. 1981; Joyce and van Hartesveldt 1984) that circling is elicited by injections of DA into the dorsal STR but not by infusions into the ventral STR. Dunnett and associates (1981a, b) have also suggested that the dorsal anterior STR critically subserves rotation. They reported that functional recovery, as measured by attenuation of amphetamine-induced circling, following embryonic grafts is dependent upon the locus of the graft. Specifically, rotation was attenuated following dorsal anterior STR grafts but not after transplants which reinnervated the ventral or ventrolateral STR.

While Joyce and van Hartesveldt (1984) have suggested that circling is subserved specifically by the medial dorsal STR, the present results indicate that DA metabolism is augmented throughout the dorsal anterior STR by conditioned circling. The medial and lateral regions are, however, differentially affected. It is possible that the changes observed within the anterior dorsomedial STR are related specifically to the rotational response exhibited. In contrast, those changes detected within the anterior dorsolateral STR, the region posited by Joyce and associates to subserve sensorimotor functioning, may reflect the involvement of sensorimotor functions in the performance of the conditioned circling response. If this is the case, animals tested in the conditioned circling paradigm might be expected to exhibit enhanced reactivity to sensory stimulation. Alternatively, the changes noted in the anterior dorsolateral region may also be a direct consequence of the rotational response involved in *conditioned* circling.

It is noteworthy that Dunnett and Iversen (1982) have failed to observe any effect of locus of discrete striatal lesions on the occurrence of subsequent drug-induced circling. Rather, similar magnitudes and directions of circling were observed with each lesion site. It is possible that the precise neural substrate of circling is dependent upon the specific procedures used to elicit circling. If this is the case, further experimentation will be required to determine how these responses differ both behaviorally and neurochemically.

While it has been reported that striatal DA metabolism is selectively increased in the contralateral STR of rats trained to circle (Yamamoto and Freed 1982), no evidence of an asymmetrical increase in DA metabolism was obtained in the second experiment. The bilateral nature of the present neurochemical effects is of particular interest given that the animals were trained to circle in the direction consistent with their innate directional bias, a procedure which should enhance the likelihood of detecting asymmetrical changes in DA metabolism. For example, the direction and intensity of circling exhibited following unilateral 6-OHDA lesions of the substantia nigra is dependent upon whether the "dominant" is lesioned (Robinson and Becker 1983). It is also important to note that the increases detected in each STR occurred within comparable regions. Accordingly, there was no evidence of an antagonistic interplay between striatal subregions (Vaccarino and Franklin 1984). Accordingly, the present results confirm earlier reports of a bilateral involvement of the mesotelencephalic DA system in conditioned circling (Schwarting and Huston 1987; Szostak et al.1986). Further support for the bilateral involvement of this projection has been provided by

the observation that conditioned circling is attenuated following unilateral lesions of the mesotelencephalic DA projection (Reite et al. 1986; Szostak et al. 1988b). However, while deficits in ipsiversive and contraversive conditioned circling are observed following a unilateral lesion, the extent of the deficit is dependent upon the locus of the lesion with respect to direction of reinforced circling. Accordingly, these studies suggest that conditioned circling is subserved by a bilateral, although asymmetrical, involvement of the mesotelencephalic DA projection. Detection of the underlying asymmetry may be dependent upon the experimental parameters and neurochemical methods employed.

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